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The Vitamin D and Provitamin D Content of Some Varieties of Utah Wheat

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THE VITAMIN D AND PROVITAMIN D CONTENT OF SOME VARIETIES OF UTAH WHEAT

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"Would that you could meet the sun and
the wind with more of your skin and less
of your raiment,

For the breath of life is in the sunlight
and the hand of life is in the wind."

"The Prophet", by Kahlil Gibran

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Cereal grains were known to be notably deficient in the antirachitic principle even before the discovery of vitamin D. Clinicians had associated a type of rickets of infancy, characterized by an overweight, flabby, bowlegged condition, with a dietary consisting mainly of cereals. Mellanby (1) in 1921 concluded that this type of rickets is induced by overfeeding with carbohydrates. He also showed in the same report, however, that oatmeal, although it contains less carbohydrates than the other grains tested, possesses a special tendency toward rachitogenesis.

In 1925, after a more thorough study of the problem, Mellanby (2) stated that, not only did the cereals studied fail to contain the antirachitic substance, but they definitely contain some substance which is inimical to the antirachitic factor. He stated in this report that oatmeal, maize, barley, rise and wheaten flour were deleterious in this respect in the order named. Later (3) he describes methods of overcoming the effects of the anticalcifying substance. These methods were (a) boiling in one per cent HCl and neutralizing with NaOH, and (b) germination followed by heating at 100°C for 18 hours. Mellanby suggested that the anticalcifying factors be called "toxamins".

Steenbock, Black and Thomas (4) in 1927 compared the rachitogenic properties of corn, wheat and rolled oats. They were unable to substantiate the findings of Mellanby. They found the antirachitic potency to rank in this order: wheat, rolled oats, and corn. Ir-

radiation corrected the difficulties of calcification and made the cereals approximately alike in their antirachitic properties. These workers would not admit the existence of an "anti-vitamin".

Mirvish (5) in 1929 extracted a substance from oatmeal which, when injected into rabbits, lowered the blood Ca 30 to 35 per cent in twenty-four hours, with a return to normal in about forty-eight hours.

De Weldt and Brouwaer (6) compared corn, barley, rye, wheat and oatmeal by feeding the grains to rats noting their effects on bone formation and composition. They used 76 per cent of each of the grains along with 20 per cent gluten, 3 per cent $CaCO_3$ and 1 per cent NaCl. They found corn to be the worst and barley the best while wheat and rye occupied intermediary positions from the point of view of the production of rickets in rats. The effect of oatmeal was uncertain as the rats on that diet failed to make sufficient growth.

Wheat ranks among the highest in antirachitic potency. The ash and protein contents of wheat have been shown to be functions of the variety. Greaves and Anderson (8) have found variety to be the principel factor governing the copper content of wheat. The question, therefore, arises as to whether or not any difference exists in the vitamin D and/or provitamin D content of varieties of wheat. It was in an effort to answer this question on a relative basis that this work was undertaken. The general plan for attacking the problem was as follows: The wheats used were grown on the same soil and under similar conditions. During harvesting, threshing, and storage the wheats were all handled in the same manner, consequently differences found

would be due to differences inherent in the different varieties. The vitamin D content was determined by feeding the different varieties of non-irradiated wheat and noting the degree of healing effected. The provitamin content was determined by feeding irradiated wheat of the same varieties to rachitic rats and measuring the degree of healing which took place upon this treatment.

Young rats of from four to five weeks of age and weighing from 45 to 65 grams, were used in the experiment. The animals were kept in individual cages in a darkened room. They were made rachitic by feeding the Steenbock diet No.2965 for 21 days or until a mild degree of rickets developed. The degree of rickets was determined by sacrificing one or more animals of each group and making the line test (10), and determining the inorganic phosphate content of the blood plasma. The proper rachitic state was indicated when the provisional line of calcification had completely disappeared from the epiphyseal juncture of the proximal end of the tibia. When this state was reached, the remaining rats were kept another five days on the rachitogenic diet. During this period the Steenbock diet was supplemented by the varieties of wheat to be tested. The ground wheat was fed in shallow dishes on three levels: 0.75, 1.00, and 1.25 grams in the case of non-irradiated; and 0.25, 0.50, and 0.75 grams in the case of the irradiated. The wheats were irradiated by exposing in thin layers to the rays of a mercury arc lamp for thirty minutes at a distance of 16 inches. The positive control groups (those in which the development of rickets was prevented) were given one drop of cod liver oil every other day. This was in addition to the Steenbock diet. The negative control groups (those in which rickets had

developed) received only the rachitogenic ration.

Records were kept of the food consumed and the gains in weight made. All animals made satisfactory gains. The average gains in weight of the rats are shown in Table 1.

Table 1

SUMMARY OF GAIN IN WEIGHT OF RATS ON VITAMIN ASSAY

Time: 26 days.

Variety	Non-irradiated			Irradiated		
	Aver. Initial Wt.	Aver. Final Wt.	Aver. Gain	Aver. Initial Wt.	Aver. Final Wt.	Aver. Gain
Baart	53.0	72	19	57	80	23
Alton	54	74	20	59	84	25
Kharkov	56	73	17	60	84	24
Kofod	58	92	34	58	81	23
Sevier	59	76	17	58	74	16
Regal	59	75	16	58	79	21

On the fifth day the rats were anesthetized and bled. The blood was analyzed for inorganic phosphate by the method of Youngberg with the modification suggested by Kuttner and Lichtenstein (11). The line test was made on the right tibiae.

A preliminary study was made to determine the levels of dosage of wheat to be fed. Six animals were used in each group. The calcium:phosphorus ratio of the Steenbock diet was found to be 4.1:1. The change in the Ca:P ratio resulting from the addition of the wheat to the diets is calculated from the average consumption of rachitogenic diet and wheat by each group. These data are exhibited in Table II. The percentage

of phosphorus in the diets eaten by these rats averaged between 0.27 and 0.30 per cent.

Table II

	Non-Irradiated Wheat				Irradiated Wheat			
	.75g	1.0g	1.25	5.0g	.25g	.50g	.75g	1.00g
Aver Line Test	.17-	.67-	1.00-1.0	1.0-	S	1.00-	1.00-	1.67-
Aver. Ca:P Ratio	3.8	3.6	3.5	1.6	3.95	3.8	3.65	3.65
Mgms. % P in plasma	2.32	2.87	2.76	3.26	1.65	2.69	2.90	3.68

The production of experimental rickets in rats is dependent not only upon the lack of vitamin D in the diet, but also upon an unbalance of calcium and phosphorus. The ratios of Ca:P preferred is that of about 4:1. Querido (12) has shown too that the percentage of phosphorus in the diet is a factor in determining the amount of vitamin D required to prevent the development of rickets. His experiments indicate that on diets in which the percentage of phosphorus is low, (0.12%) the amount of vitamin D required to prevent rickets increases as the Ca:P ratio decreases. With a higher percentage of phosphorus (0.35%) the reverse is true. When the Ca:P ratio is 4 and the percentage phosphorus is 0.35 per cent mild rickets develop. This condition of rickets is preventable by minute doses of vitamin D. In view of these facts it is unsafe to attribute to the vitamin D content of the wheat the healing observed on feeding five grams of wheat per day. When this amount was

fed the Ca:P ratio dropped to 1.6. For this reason this level of dosage was omitted in later experiments. These facts, however, do raise the question as to the limits to which the calcium:phosphorus rationand the absolute phosphorus intake can go without changing significantly the amount of vitamin required to promote healing.

Table III shows the average daily Ca and P intake and the Ca:P ratio of diets when the rats were receiving the wheat and Steenback diet.

Table III

THE CA:P RATIO IN THE DIETS, AND THE DAILY Ca AND P INTAKE WHEN NON-IRRADIATED WHEAT WAS FED

Variety of Wheat	Amt. Wheat fed per day gms.	Aver. daily Ca. intake mgms.	Aver. Daily P. intake mgms.	Ca:P ratio
Baart	0.75	101	26	3.9
	1.00	89	24	3.7
	1.25	76	21	3.6
Alton	0.75	101	26	3.9
	1.00	102	27	3.8
	1.25	102	27	3.8
Kharkov	0.75	127	32	4.0
	1.00	76	21	3.6
	1.25	76	21	3.6
Kofod	0.75	101	26	3.9
	1.00	89	24	3.7
	1.25	89	24	3.7
Sevier	0.75	89	23	3.9
	1.00	89	24	3.7
	1.25	89	24	3.7
Regal	0.75	114	29	3.9
	1.00	102	27	3.8
	1.25	51	15	3.4

Table IV

INORGANIC PHOSPHATE IN BLOOD PLASMA, ESPRESSED IN MGMS. PER 100 C.C. PLASMA

	Non-Irradiated			Irradiated		
Variety	.75g	1.00g	1.25g	.25g	.50g	.75g
Baart	2.32 ± .16	2.87 ± .14	2.76 ± .13	1.94 ± .03	2.69 ± .05	2.90 ± .16
Alton	1.93 ± .04	2.31 ± .04	2.39 ± .13	1.88 ± .04	2.74 ± .13	3.37 ± .05
Kharkov	2.27 ± .13	2.06 ± .07	2.43 ± .11	1.78 ± .03	2.18 ± .05	2.70 ± .01
Kofod	1.47 ± .05	2.67 ± .24	2.22 ± .10	1.86 ± .10	2.84 ± .03	3.08 ± .10
Sevier	1.50 ± .06	1.97 ± .03	2.02 ± .24	2.01 ± .22	2.08 ± .22	2.52 ± .33
Regal	2.00 ± .09	1.75 ± .09	2.22 ± .10	1.78 ± .09	2.78 ± .05	2.07 ± .19

Table V

AVERAGE LINE TEST

	Non-Irradiated Wheat			Irradiated Wheat		
Variety	0.75g	1.00g	1.25g	0.25g	0.50g	0.75g
Baart	0.25+	.75+	.75+	S	1.00+	1.00+
Alton	Severe Rickets	.25+	1.00+	S	1.00+	1.00+
Kharkov	0.25+	.67+	1.00+	S	S	1.00+
Kofod	S	.59+	1.00+	S	1.00+	1.00+
Sevier	S	.75+	1.00+	S	0.75+	1.00+
Regal	S	S	1.00+	S	1.00+	1.00+

As a result of this preliminary study it was decided to feed the wheats in dosages of 0.75, 1.00, and 1.25 grams of the non-irradiated, and 0.25, 0.50, and 0.75 grams of the irradiated as was previously stated.

The criteria used for measuring the antirachitic potency were the plasma phosphorus content of the blood and the recalcification of the rachitic metaphyses as indicated by the line test. The results of the former measurements are shown in Table IV, those of the latter in Table V.

The numerical values were obtained by estimating the degree of healing, from one plus to four plus, and computing the numerical average from these estimations. Severe rickets is indicated by zero.

The phosphorus content of the plasma of the positive control rats averaged 3.76 ± 0.11 mgms. per 100 c.c. of plasma, the negative control rats averaged 2.16 ± .09. The bones of the positive control animals were all normal. The negative control rats used in obtaining the average stated above were selected to include only those in which severe rickets was present.

A slight degree of healing of the rachitic lesions occurred when the non-irradiated wheats were fed in amounts of 1.25 gms. The blood phosphorus, however, did not increase significantly in comparison with the controls, except in the case when Baart was led. The line test values obtained leave some doubt as to any superiority of Baart over the other varieties studied.

The wheats were grown side by side and were presumably handled in the same manner after threshing. Solar irradiation during growth and subsequent handling could possibly have been the cause of the anti-rachitic activity observed.

The rickets-curing property was materially enhanced by irradiating the wheat. This fact is shown by both the line test and the increase in blood phosphorus. A significant difference existed in the blood phosphorus of the rats fed irradiated Baart, Alton, Kofod and Regal as compared with those fed irradiated Kharkov and Sevier.

CONCLUSIONS

1. The varieties of non-irradiated wheat tested appear to have slight antirachitic properties.

2. Irradiation increases the vitamin D content of wheat.

3. A difference appears to exist in the provitamin D contents of varieties of wheat.

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